



GROWTH FACTORS, CYTOKINES, AND CELL CYCLE MOLECULES

Increasing Dietary Selenium Elevates Reducing Capacity and ERK Activation Associated with Accelerated Progression of Select Mesothelioma Tumors

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To study the effect of the micronutrient selenium on malignant mesothelioma (MM) progression, we cultured four different MM cell lines in media containing increasing amounts of sodium selenite (30, 50, and 80 nmol/L). Increasing selenium levels increased density-dependent proliferation and mobility for CRH5 and EKKH5 but not AB12 and AK7. Comparing these cell lines revealed that extracellular regulated kinase (ERK) phosphorylation was sensitive to a selenium increase in CRH5 and EKKH5 but not AB12 and AK7 cells. Stable expression of a dominant-negative mutant ERK eliminated the effects of increasing selenium. Because ERK is redox sensitive, we compared the MM cell lines in terms of glutathione levels and the capacity to reduce exogenous hydrogen peroxide. Increasing selenium levels led to higher glutathione and reducing capacity in CRH5 and EKKH5 but not AB12 and AK7. The reducing agent *N*-acetylcysteine eliminated the effects of selenium on ERK activation, proliferation, and mobility. Mice fed diets containing increasing levels of selenium (0.08, 0.25, and 1.0 ppm) showed increased tumor progression for CRH5 but not AB12, MM cells, and *in vivo* *N*-acetylcysteine treatment eliminated these effects. These data suggest that the effects of dietary selenium on MM tumor progression depend on the arising cancer cells' redox metabolism, and the tumors able to convert increased selenium into a stronger reducing capacity actually benefit from increased selenium intake. (*Am J Pathol* 2014, 184: 1041–1049; <http://dx.doi.org/10.1016/j.ajpath.2013.12.008>)

Dietary selenium is an essential micronutrient that is important for many aspects of human health.¹ The biological effects of selenium are exerted mainly through the actions of the proteins into which it is incorporated (ie, selenoproteins).² The human genome contains 25 genes that encode for selenoproteins and several of these are enzymes that play important roles in regulating cellular redox status.^{3,4} Within cells, dietary selenium also may be metabolized into intermediate compounds such as methylselenol, which affect redox homeostasis and may exert toxic effects on cancer cells.^{5,6} An abundance of preclinical findings together with some clinical data have suggested that selenium supplementation may prove to be an affordable, effective means to prevent or treat a wide variety of cancers.^{7,8} However, the effectiveness of selenium supplementation for cancer prevention has been inconsistent and somewhat controversial.^{9–11} These inconsistencies may be owing to differences in the form of selenium supplementation used, to the baseline

selenium status of the participants in different populations, or to other study design factors.¹² In addition, certain selenoproteins have been shown to actually promote tumor progression,^{13,14} which highlights the importance of identifying the molecular mechanisms by which dietary selenium influences the development of each type of cancer.

Malignant mesothelioma (MM) is a deadly cancer associated with asbestos or erionite exposure for which no successful therapies are currently available. MM is among the most aggressive tumors, arising from the mesothelial cells that line the pleura, peritoneum, and, occasionally, the pericardium.¹⁵ Dietary selenium has been proposed to play a potential chemopreventive role in the prevention or treatment

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of MM, although the data are limited. In one study involving a MM cell line, selenium was shown to inhibit MM cell growth and induce apoptosis in a dose-dependent manner.¹⁶ However, the levels of selenium required for these effects were extremely high, and other *in vivo* studies involving selenium and vitamins A and E did not show beneficial effects of any of these dietary antioxidants on tumor development.¹⁷ Determining how selenium status affects MM tumor development and progression is essential given that MM incidence is increasing in the United States and other parts of the world,¹⁸ and selenium intake varies in different geographic regions, with selenium supplementation considered as a potential treatment approach for MM and other cancers.

An emerging perspective of the role that selenium may play in chemoprevention has focused on the different stages of tumor development: the initial carcinogenesis events involving DNA repair in which increased selenium may be beneficial, and the subsequent progression of established tumors for which the effects of increasing selenium intake may vary.¹⁹ To address the issue more specifically of whether different levels of selenium intake influence the progression of MM tumors after they have been established, we used several different MM cell lines generated by the administration of asbestos particles into mice and investigated the impact of dietary selenium levels on tumor progression. Surprisingly, we found that increasing selenium intake did not limit the growth of any of the cell lines and higher selenium levels actually promoted proliferation, mobility, and *in vivo* tumor progression for some MM cell lines. We further identified molecular mechanisms that were affected by higher selenium intake involving redox-sensitive signaling pathways converging at the point of extracellular regulated kinase (ERK) phosphorylation. Overall, the data presented herein provide crucial insight into the mechanisms by which dietary selenium affects MM tumor progression and suggest that the use of selenium supplementation to treat MM may in some cases be more harmful to the patients.

Materials and Methods

Mice, Cell Lines, and Reagents

C57BL/6 and Balb/c mice originally obtained from Jackson Laboratories (Bar Harbor, ME) were used to generate colonies. Male weanling mice were maintained on diets containing different levels of selenium (0.08, 0.25, or 1.0 ppm selenium) for a minimum of 4 weeks to effectively establish selenium status as previously described.²⁰ At 8 to 10 weeks of age, mice were used for experiments, and all animal protocols were approved by the University of Hawaii Institutional Animal Care and Use Committee. The MM cell lines AB12, AK7, CRH5, and EKKH5 were generated as previously described,^{21–23} and cultured in F-12 media with 10% fetal bovine serum (GIBCO/Invitrogen, Grand Island, NY). The selenium content of the fetal bovine serum was determined by IC-MS (West Coast Analytical Service, Santa Fe Springs,

CA), and increasing levels of selenium were established in the culture media by addition of sodium selenite (Sigma, Chicago, IL) to the complete F-12 media at a final concentration of 30 nmol/L, 50 nmol/L, and 80 nmol/L. Cells were maintained under these conditions for 1 week before experimentation to establish selenium status. The reducing agent *N*-acetylcysteine (NAC) was purchased from Sigma-Aldrich (St. Louis, MO).

GPx Activity and GSH Assays

After cells were cultured for at least 1 week in low (30 nmol/L), medium (50 nmol/L), or high (80 nmol/L) levels of selenium, cell pellets were collected, cells were lysed, and glutathione peroxidase (GPx) activity was analyzed using a Bioxytech GPx-340 Colorimetric Assay Kit (Oxis Research, Foster City, CA). Total protein content was measured using a Bradford Assay (Bio-Rad, Hercules, CA). GPx activity was normalized to total protein concentration for each sample. For glutathione (GSH) measurement, cell pellets were harvested in a similar manner and GSH levels were measured using a Bioxytech GSH-400 (Oxis Research) and normalized to protein concentration.

Cell Proliferation and Mobility Assays

After 1 week of maintenance in media with low (30 nmol/L), medium (50 nmol/L), or high (80 nmol/L) levels of selenium, cell lines were plated at a density of 10^3 cells/well in a 96-well plate in 200 μ L fresh media. Media were replaced every 24 hours to prevent selenium depletion. Every 24 hours cells were lysed and quantified using Cyquant Fluorescent Dye (Invitrogen) and fluorescence detection on a Molecular Devices Spectramax M3 (Sunnyvale, CA). After reaching 100% confluency the Cyquant Fluorescent Dye would not accurately bind to target DNA, which led to extremely high and fluctuating fluorescence readings. Thus, both AK7 and AB12 reached confluency at day 6, EKKH5 at day 7, and CRH5 at day 8, and Cyquant dye incorporation was measured up until those points. After cells were cultured for 1 week in low (30 nmol/L), medium (50 nmol/L), or high (80 nmol/L) levels of selenium, 10^6 cells/well were plated in 6-well plates for 24 hours before performing the scratch assay. A 200- μ L pipette tip was used to create four vertical scratches in the adherent monolayer. The cells were washed with PBS and then covered with 2 mL of low, medium, or high selenium media. A Zeiss Axioscope (Oberkochen, Germany) with a mounted camera was used to capture images for 10 fields from each cell line in each condition at 0 and 6 hours after scratch. The images then were analyzed using ImageJ software version 1.42 (NIH, Bethesda, MD) to compare the change in area of the scratch at each point.²⁴

Western Blots and Real-Time PCR

Cell pellets were harvested and lysed in CellLytic MT buffer (Millipore, Billerica, MA) using a probe sonicator. In the

case of measuring phosphorylated AKT (pAKT) and phosphorylated ERK (pERK), cells were cultured in 0.2% fetal bovine serum media for 24 hours before protein isolation. Protein concentration in the lysates was measured by a Bradford assay reagent (Bio-Rad) and 15 µg total protein was combined with reduced Laemmli buffer, boiled at 95°C for 10 minutes, cooled on ice, and loaded into wells of 10% to 14.5% polyacrylamide gels (Bio-Rad). After gel electrophoresis, protein was transferred to polyvinylidene difluoride membranes, which were blocked for 1 hour with low fluorescence blocking agent (Li-Cor, Lincoln, NE) and incubated with primary antibodies including anti-pAKT, anti-total AKT, anti-pERK, anti-total ERK (Cell Signaling, Inc., Danvers, MA), anti-GPx1 (Lab Frontier, Inc., Seoul, Republic of Korea), or anti-β-actin (Li-Cor). After washing with PBS, membranes were incubated with secondary antibodies from Li-Cor for 1 hour, membranes were washed with PBS, and signals were detected with densitometry conducted using the Li-Cor Odyssey imaging system. Real-time PCR was performed as previously described²⁵ using the following primers: c-fos forward 5'-CTCCCGTGGTCACCTGTACT-3', c-fos reverse 5'-TTGCCTTCTCTGACTGCTCA-3', actin forward 5'-TGACAGGATGCAGAAGGAGA-3', and actin reverse 5'-CGCTCAGGAGGAGCAATG-3'.

Subcutaneous Tumor Growth

Weanling mice were fed low (0.08 ppm), medium (0.25 ppm), and high (1.0 ppm) selenium diets for 4 weeks. These mice were then injected s.c. with 10⁶ AB12 or CRH5 MM cells. The mice were monitored for 6 to 7 weeks and tumor volume was measured using digital calipers as previously described.²¹ The mice were sacrificed when tumors reached volumes that threatened the health of the mice (volume, >1500 mm³).

Stable Transfection

The CRH5 and EKKH5 cell lines were transfected with a plasmid encoding the dominant-negative form of ERK (TAYE-ERK; a kind gift of Dr. James Turkson, University of Hawaii Cancer Center) and Lipofectamine reagent (Invitrogen). Stably transfected cells were selected by culturing cells in media containing G418 (Sigma). A killing curve of 100 to 1000 µg/mL showed that 300 µg/mL was the optimal dosage for selection; G418 was removed after the TAYE-ERK CRH5 and EKKH5 cell lines were established.

Statistical Analysis

GraphPad Prism software version 4.0 (GraphPad, La Jolla, CA) was used to compare means in experiments with two groups using the Student's *t*-test. In assays involving three groups of selenium levels, a one-way analysis of variance was used to determine the effect of selenium on outcomes, using the Tukey post-test to compare means of selenium in groups. For the proliferation assays involving selenium levels and time, a two-way analysis of variance was used to test the main effects of selenium and time on outcomes.

In addition, interactions between selenium and time on proliferation were analyzed. The Bonferroni post hoc test was used to identify the means of different selenium groups that differed. Standard curves and regression analyses also were conducted using GraphPad Prism software version 4.0. All comparisons were considered significant at a *P* value less than 0.05.

Results

Certain MM Cell Lines Derive a Proliferative and Migratory Advantage from Increasing Selenium Concentration

MM cell lines AB12, AK7, CRH5, and EKKH5 were cultured in media containing increasing levels of selenium.

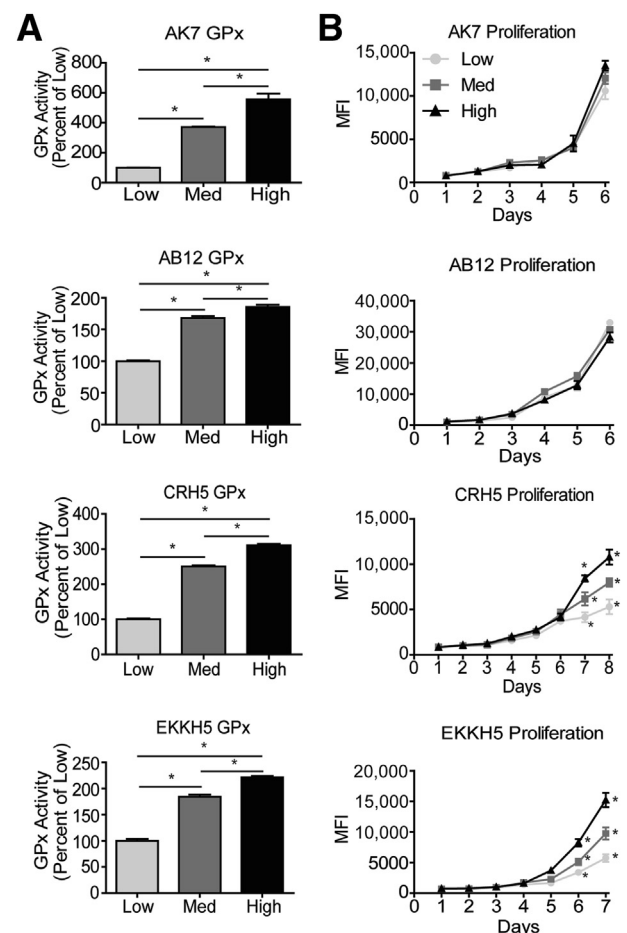


Figure 1 Increasing selenium levels increases the proliferation of select MM cell lines. Cellular GPx activity increases with increasing selenium levels [low (30 nmol/L), medium (50 nmol/L), and high (80 nmol/L)] in all MM cell lines (AK7, AB12, EKKH5, and CRH5). **A:** A total of 10³ cells/well were plated and allowed to grow to confluency with media changes daily. **B:** Proliferation of AK7 and AB12 cells was not affected by selenium levels, whereas CRH5 and EKKH5 cells showed an increase in proliferation with increasing levels of selenium during the late stages of growth. Data represent means ± SEM. *N* = 3. Means of each selenium group were compared at each time point. Each experiment was repeated at least twice. **P* < 0.05. MFI, mean fluorescence intensity.

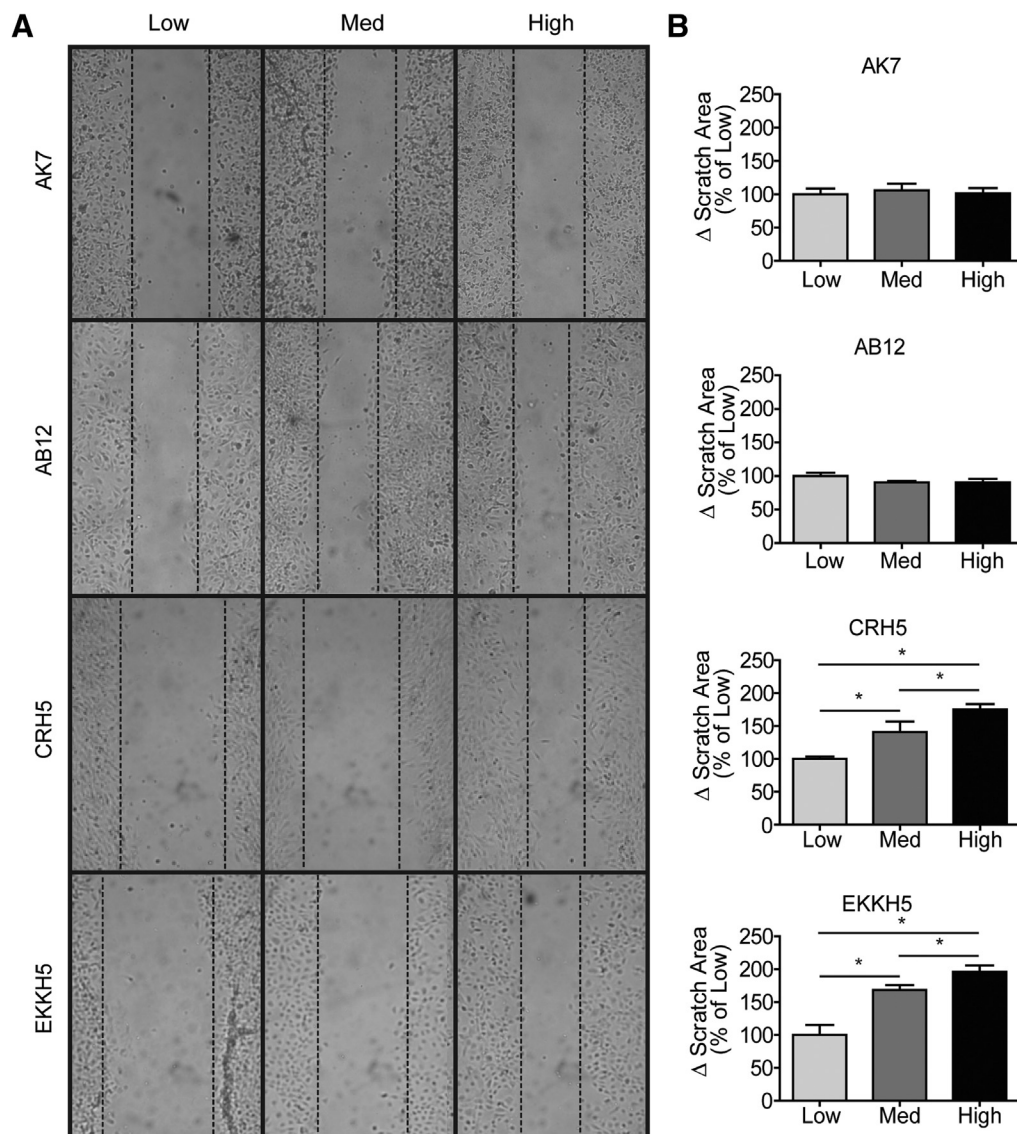


Figure 2 Increasing selenium levels affect the mobility of CRH5 and EKKH5 MM cell lines similar to proliferation. Four different MM cell lines were cultured in media containing 30, 50, and 80 nmol/L selenium for 1 week, cells were replated, and allowed to grow to confluency. Each experiment was repeated twice. Images from the scratch assays captured at 0 and 6 hours show mobility. **A:** Representative images at 6 hours are shown. **B:** Quantification of the scratch volume was calculated by ImageJ software. Data represent means \pm SEM. * $P < 0.05$.

Activity of the selenoenzyme, GPx, was measured to confirm the biological effects of increasing selenium in the media, and the effects of increasing selenium on proliferation then was evaluated (Figure 1A). Although all cell lines equivalently used the bioavailable selenium for increasing GPx activity, the effect of selenium levels on proliferation differed among cell lines (Figure 1B). The MM cell lines CRH5 and EKKH5 showed two stages of growth characterized by an early selenium-independent phase and as the cells approached confluency they showed higher proliferation with increasing selenium concentration. In contrast, proliferation of AK7 and AB12 cell lines were not influenced by increasing selenium levels. We next evaluated the MM cell lines for the effects of selenium levels on another important tumor cell function, mobility, using a standard

scratch assay (Figure 2). Similar to the proliferation data, CRH5 and EKKH5 showed increased mobility with increasing selenium whereas the mobility of the AK7 and AB12 cell lines were not affected by increasing selenium.

ERK Phosphorylation Increases with Increasing Selenium in the CRH5 and EKKH5 MM Cell Lines

To investigate signaling pathways affected in the selenium-sensitive versus selenium-insensitive MM cell lines, we evaluated two important pro-growth signaling molecules associated with proliferation and mobility in mesothelioma: ERK and AKT.^{26–28} CRH5 and EKKH5 cells that responded to higher levels of selenium with increased proliferation and mobility also were shown by Western blot analyses to

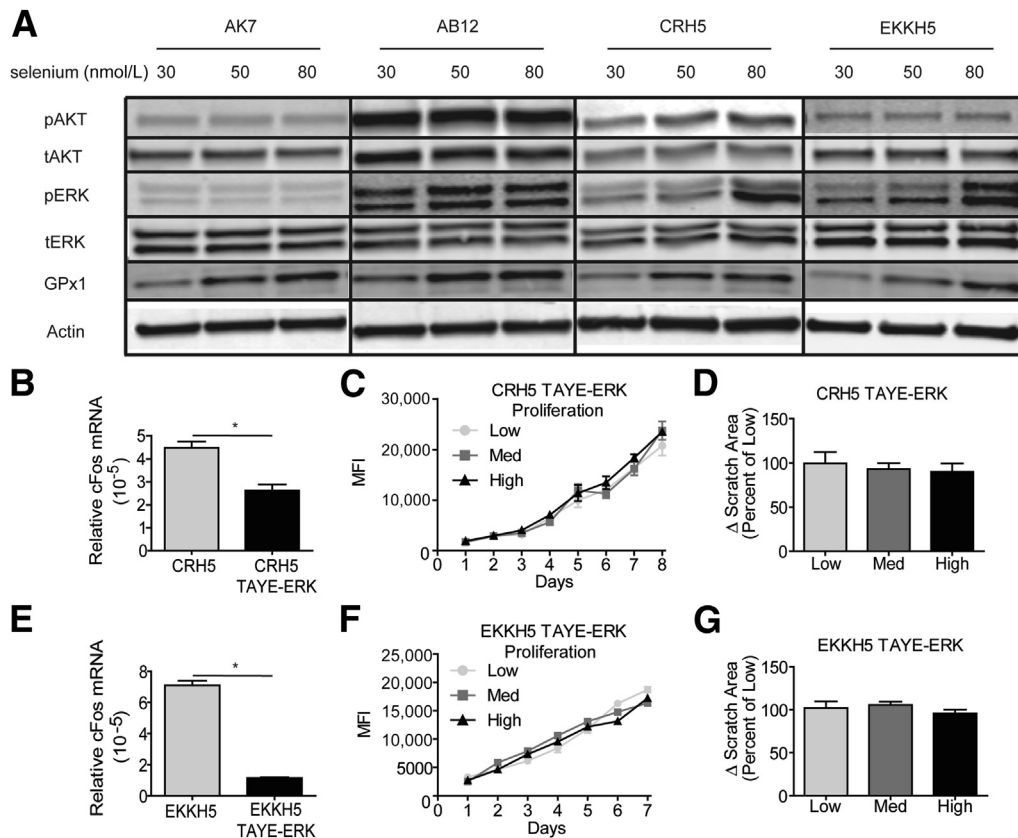


Figure 3 The effects of selenium on the proliferation and mobility of MM cell lines are mediated by ERK activation. **A:** Western blot analysis on cell lysates from 1-week-old cells show an increase in pERK levels with increasing selenium [low (30 nmol/L), medium (50 nmol/L), and high (80 nmol/L)] in CRH5 and EKKH5, but not AK7 and AB12, cell lines. CRH5 also showed a slight increase in pAKT with increasing selenium. Total ERK and AKT levels were equivalent, as was β -actin (loading control). Levels of GPx1 confirmed equivalent use of bioavailable selenium for each MM cell line. Data are representative of two independent experiments. C-fos mRNA levels (**B** and **E**), mean fluorescence intensity (MFI) (**C** and **F**), and scratch area (**D** and **G**) in CRH5 and EKKH5 cells are shown. The cells were stably transfected with a plasmid encoding a dominant-negative form of ERK (TAYE-ERK). Levels of c-fos mRNA were compared with housekeeping β -actin mRNA for each sample using real-time PCR. The TAYE-ERK CRH5 and TAYE-ERK EKKH5 cells were cultured in media containing 30, 50, and 80 nmol/L sodium selenite for 1 week and the mobility and proliferation were evaluated. Each experiment was repeated twice. Data represent means \pm SEM. * $P < 0.05$.

have increased pERK with increasing selenium (Figure 3A). The cell lines that did not functionally benefit from increased selenium uptake, AK7 and AB12, did not show changes in pERK with increasing selenium. Only CRH5 cells showed slightly increased pAKT with increasing selenium levels, whereas selenium levels did not affect pAKT in the other cell lines. Because the effects of increasing selenium on proliferation and mobility shown earlier correlated most consistently and clearly with ERK activation, we next generated CRH5 and EKKH5 cell lines stably expressing the ERK dominant-negative TAYE (CRH5-TAYE and EKKH5 TAYE). The effects of expressing the dominant-negative ERK in the CRH5-TAYE and EKKH5 TAYE cell lines were confirmed because these cells showed about 40% and 15% lower levels of mRNA, respectively, for the pERK target gene, *cFos* (Figure 3, B and E). Unlike the CRH5 and EKKH5 cell lines, the CRH5-TAYE and EKKH5-TAYE cell lines showed no differences in either proliferation (Figure 3, C and F) or mobility (Figure 3, D and G) in response to increased selenium. Overall, these

data suggest that ERK activation represents an important signaling event in mediating the effects of increasing selenium in those MM cells that are responsive to changes in levels of this micronutrient.

Selenium Affects Certain MM Cells by Increasing Reducing Capacity

Because the phosphorylation of ERK has in some cases been shown to be a redox-sensitive event, we next investigated the role of redox status in the effects of increasing selenium on MM cell growth and proliferation. CRH5 and EKKH5 cells showed increasing levels of reduced GSH with increasing selenium levels, whereas AB12 and AK7 did not (Figure 4A). To determine if the reducing equivalents of GSH were used differently by the selenium-sensitive and selenium-insensitive cells, 250 mmol/L H_2O_2 was added and reactive oxygen species (ROS) levels were evaluated using dihydrochlorofluorescein. Results showed that increasing selenium levels decreased ROS levels in

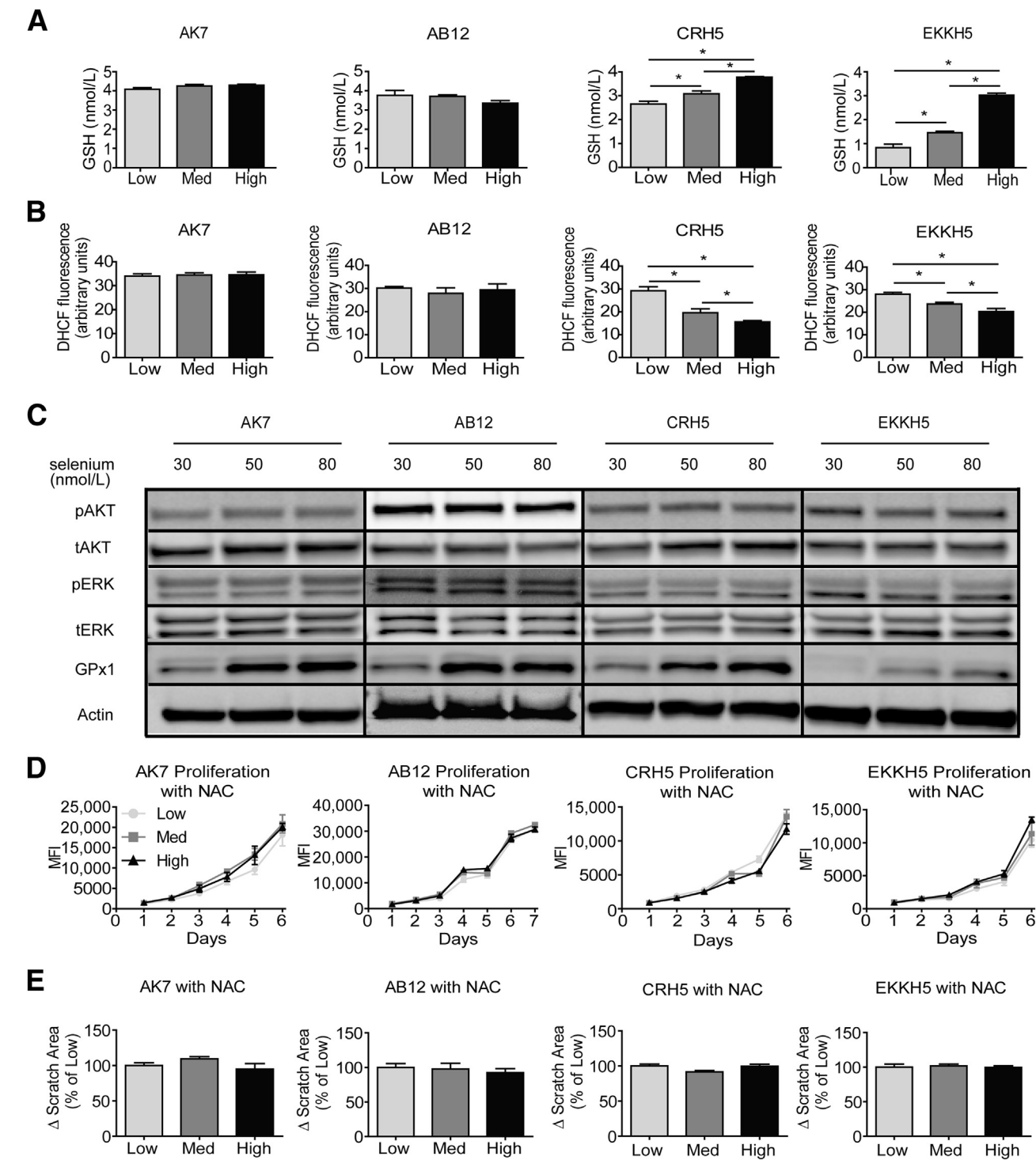


Figure 4 Reducing capacity with increasing selenium is a crucial mechanism by which proliferation and mobility are affected in selenium-sensitive cells. Cellular GSH (A), ROS levels (B), and Western blot analysis (C) in MM cell lines cultured in media containing low (30 nmol/L), medium (50 nmol/L), and high (80 nmol/L) selenium for 1 week. For ROS levels the cells were challenged with 250 mmol/L H_2O_2 . For Western blot analysis 0.4 mmol/L NAC was added as a reducing agent for 14 hours before protein extraction and Western blot analysis. No differences were found in pERK or pAKT for any of the cell lines, and levels of GPx1 confirmed equivalent use of bioavailable selenium for each MM cell line. Data are representative of two independent experiments. All four MM cell lines cultured in increasing selenium with NAC added as described earlier showed no differences in proliferation (D) or mobility (E). Each experiment was repeated twice. Data represent means \pm SEM. $*P < 0.05$.

CRH5 and EKKH5, but not in AB12 and AK7 (Figure 4B). No differences in basal ROS levels (ie, no H_2O_2 added) were found with increasing selenium levels in the MM cell lines (Supplemental Figure S1). To determine if this

difference in reducing capacity contributed to effects of selenium on ERK activation and proliferation/mobility, we used the reducing reagent NAC to eliminate differences in redox status and compared all four MM cell lines. Western

blot analyses showed that treatment with NAC led to equivalent pERK and pAKT levels in all four MM cell lines, regardless of selenium levels (Figure 4C). NAC treatment also resulted in equivalent proliferation and mobility in the MM cell lines regardless of selenium levels (Figure 4D).

In Vivo CRH5 MM Tumor Progression Increases with Increasing Dietary Selenium in a Manner that Depends on Reducing Capacity

Balb/c mice were fed defined diets containing 0.08, 0.25, and 1.0 ppm selenium as sodium selenite for 4 weeks to establish low, medium, and high selenium status, respectively, as previously described.²⁰ These mice then were injected subcutaneously with the selenium-sensitive (CRH5) and selenium-insensitive (AB12) MM cell lines. The mice were maintained on the selenium diets throughout the study and tumor volume was monitored over time. Similar to the *in vitro* data described earlier, CRH5 MM tumor progression was increased with increasing dietary selenium levels, whereas AB12 MM tumors showed no significant effect from increasing dietary selenium levels (Figure 5). NAC treatment throughout the tumor measurements eliminated the effects of dietary selenium on CRH5 tumor progression. Thus, together with the *in vitro* data described earlier these results suggest that certain MM tumors benefit from increasing selenium levels owing to increased reducing capacity exerted by this micronutrient.

Discussion

Selenium has been a topic of great interest in the field of cancer research, but the role of this essential micronutrient in mesothelioma disease onset or progression has not been elucidated fully. Because of its antioxidant and cancer-cytotoxic properties, supernutritional levels of selenium are thought to limit the pro-oxidant conditions that may initiate carcinogenesis or fuel progression of established tumors.²⁹ The role that higher selenium uptake plays in promoting DNA damage repair is very different from the role selenium and selenoproteins play in redox homeostasis in established, proliferating cancer cells.¹⁹ Although our study does not address MM carcinogenesis, our findings suggest that higher selenium intake is not beneficial for individuals with established MM tumors. In fact, depending on the manner in which increasing selenium is used for reducing capacity by the MM cells comprising the tumor, selenium supplementation may even promote tumor progression.

Mesothelioma is a relatively rare form of cancer, but significant numbers of asbestos-exposed individuals in the United States are still at risk of developing this deadly disease and the incidence of mesothelioma is increasing in many parts of the world.^{30,31} It is important to fully characterize the relationship between dietary selenium and mesothelioma given that dietary selenium intake varies widely compared with other micronutrients, with mean values of 40

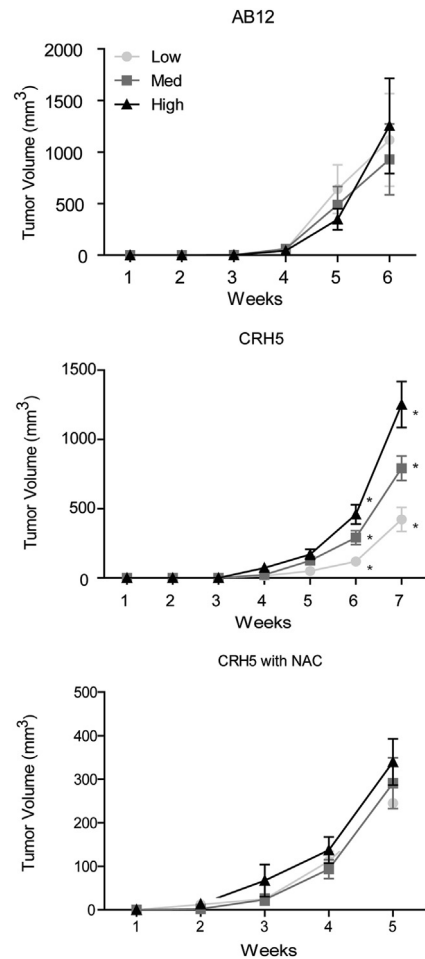


Figure 5 Increasing dietary selenium *in vivo* promotes redox-dependent tumor progression of CRH5, but not AB12, MM cells. Male Balb/c mice were fed defined diets containing low (0.08 ppm), medium (0.25 ppm), and high (1.0 ppm) selenium as sodium selenite for 4 weeks, and then 10^6 AB12 or CRH5 MM cells were injected s.c. and tumor volume measurements were performed weekly. CRH5 also was injected into mice maintained on drinking water containing 0.4 mmol/L NAC for the remainder of the experiment. Mice were sacrificed when tumor volumes threatened the health of the mice. Each experiment was repeated twice. Data represent means \pm SEM. Means of each selenium group were compared at each time point. $N = 5$ per group. * $P < 0.05$.

μg per day in Europe and 93 μg per day (in women) to 134 μg per day (in men) in the United States.¹ Furthermore, an estimated 18% to 19% of adults in the United States use supplements containing selenium³² and selenium fortification of foods is being pursued in many parts of the world to boost suboptimal selenium levels in populations and as a supernutritional means of nutritional cancer prevention.^{33–35} The minimum selenium intake and supernutritional levels in rodents has been established to be 0.1 ppm and approximately 0.8 ppm, respectively.³⁶ Thus, our study was designed to include moderately low (0.08 ppm), adequate (0.25 ppm), and supernutritional (1.0 ppm) levels of selenium intake. Some rodent anticancer studies use diets with 2.0 ppm selenium,³⁷ which is likely to further increase the levels of potential anticancer selenium metabolites.⁵

If we had increased our mouse diets to 2.0 ppm we may have found some toxic effects on the MM tumors, but given that this level is 20-fold higher than the minimum intake and the recommended 200 µg/day selenium-supplemented levels in humans is fourfold higher than the minimum intake,³⁶ we chose a more realistic value of 1.0 ppm as a supernutritional selenium diet for this study.

The specific mechanisms by which increased selenium affects cancer cells are complex, with both selenoproteins and selenium metabolites playing important roles.²⁹ In fact, selenoproteins themselves may play roles in both preventing and promoting different types of cancer.^{13,38} Our findings suggesting that some MM cells use increasing selenium to increase reducing capacity reflected by increased GSH stores are difficult to explain through a simple model of increased selenoprotein activity. There is no evidence to date showing that selenoproteins directly contribute to increased synthesis or stability of GSH, although other studies have shown interactions between increasing selenium and GSH levels.^{20,39} Comparing CRH5 with AB12 MM cell lines, both showed increased GPx1 activity with increasing selenium uptake, but only CRH5 showed increased GSH levels as well. This endowed CRH5 MM cells with the ability to detoxify H₂O₂ more effectively with increasing selenium, presumably through GPx1 activity. Thus, GSH appears to be the limiting factor in mediating the effects of increased selenium on redox status in MM cells and this further affects the activation of pro-growth effector molecules such as ERK. ERK is a crucial mitogen-activated protein kinase that previously was identified as a ROS-sensitive survival signaling factor.⁴⁰ It also has been shown that ERK activation is an essential step in the progression of mesothelioma tumors.⁴¹ Thus, we have established an important link between selenium uptake, GSH reserves, and ERK activation that may reflect a different metabolism that is established in cancer cells arising from inflammation driven by the original exposure to asbestos or erionite. AKT was found to change with increasing selenium levels only in CRH5, and this pro-growth factor may reflect an additional signaling factor involved in the effects of selenium on growth for some tumor cells, warranting further investigation.

Interestingly, our findings suggest that MM cells, which use bioavailable selenium more efficiently for higher reducing capacity, may better adapt to the oxidative stress associated with the dense environment of tumors. The data showed that CRH5 and EKKH5 cells benefitted most from higher selenium conditions as cells approached confluency. Although our data do not definitively show a role for reducing capacity or ERK activation in directly mediating the effects of selenium on tumor cell growth, they do support the notion that redox tone and ERK activation are linked to selenium levels and are involved in the proliferation of some MM cells. Our data are consistent with the observations that the need of cancer cells to detoxify ROS increases as the cell density increases.⁴² Whether MM cells

use increasing selenium for increasing antioxidant capacity may depend on their location within a tumor, availability to the vasculature, or other factors. Overall, our data suggest that increasing intake of selenium may not be beneficial for MM patients and provide important insight into the use of this micronutrient by certain MM cells for increasing proliferative capacity as cells approach high-density conditions.

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Supplemental Data

Supplemental material for this article can be found at <http://dx.doi.org/10.1016/j.ajpath.2013.12.008>.

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